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Perspective Article

Hylonome

VEGF and bone

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The role of the vasculature in osteogenesis was appreciated as early as 1763, when Albrecht von Haller suggested that blood vessels are responsible for bone formation[1]. In this same time period, John Hunter noted the importance of the vasculature during bone development and following bone damage[2]. Interest in the vasculature of bone was revived in 1963 with the proposal of a "vascular stimulating factor (VSF)" present and active at sites of bone injury[3].

Time has shown the accuracy of these early predictions. Decreased bone formation and bone mass are associated with insufficient or inappropriate blood vessels in/around bone[4,5], and 5-10% of the 6 million U.S. bone fractures/year do not heal normally[6,7]. In animal models, inhibition of angiogenesis leads to a histological picture similar to that of human atrophic non-unions[8]. Clinically, risk factors for delayed or non-unions – such as inadequate immobilization, drug use, advanced age, diabetes and poor nutrition[7] - may be associated with inadequate blood flow or injury site vascularity, suggesting that adverse effects on the vasculature may be a mechanism by which at least some risk factors impair human bone healing[5].

Fracture healing occurs in four main phases[7,9-12]. First, a hematoma is formed and seals off the fracture area. The hematoma is important for healing, as its removal significantly inhibits healing. The hematoma can induce bone formation at a new site[13,14], consistent with its inherent angiogenic properties[15,16]. In this phase of healing, inflammatory cells, fibroblasts, and stem cells are recruited, and growth factors and cytokines are released into the wound site[7,10,11,17,18]. During these early steps, angiogenesis – or the formation of new blood vessels form from pre-existing ones – occurs. Subsequently, fibrocartilage replaces the granulation tissue at the ends of the bones, and the external callus is formed by intramembranous ossification at the periosteum. Next, a hard callus of woven bone is formed as the internal callus becomes mineralized. In the final stage of bone repair, secondary lamellar bone replaces woven bone, and the callus and vasculature revert to normal shape and size. This pattern of human bone healing can be recreated in animal models of bone injury[7-12,19-21].

Many of the steps of bone formation during development also occur during bone repair. Accordingly, many of the same molecules – such as members of the fibroblast growth factor (FGF), transforming growth factor-beta (TGF-β), bone morphogenetic protein (BMP), insulin-like growth factor (IGF), and platelet-derived growth factor (PDGF) families and vascular endothelial growth factor, VEGF - are expressed and active during both development and repair[5,7,9-11,15-18,20-24]. Other pro-[11] or anti-angiogenic molecules expressed in the growth plate during development[22] may play a role in fracture repair. Tissue damage is followed by both local and systemic angiogenic responses[5,11,15,16]. Accordingly, many systemic factors can affect bone formation and vascularity[7,10].

Endogenous VEGF is important for bone formation. In developing mice, blocking VEGF activity results in an enlarged area of hypertrophic cartilage, loss of metaphyseal blood vessels, and impaired trabecular bone formation[24-26]. Thus, during development, VEGF is essential for normal growth plate morphogenesis, including blood vessel invasion and cartilage remodeling[24-26]. VEGF has also been implicated in bone repair[19,27-29]. During bone repair, VEGF is expressed in a similar pattern as that which occurs in development[11,20,21]. The angiogenic activity of the hematoma (which does not exist during development) and the plasma from injured individuals are due primarily to VEGF[15,16]. Inhibition of VEGF activity delays bone repair in mice[19] and decreases blood flow and leads to non-unions in rabbits[27]. Thus, during bone repair, VEGF is required not only for blood vessel formation, but also for normal callus volume and mineralization. These results indicate that normal angiogenesis is central to tissue repair, and that VEGF may be the major signal to couple angiogenesis and osteogenesis during bone repair[19,27-29].

VEGF may be at least one of the mediators of growth fac-

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tor stimulation of bone repair in animal models and clinical trials. Most osteo-inductive growth factors, as well as ultrasound[30], induce VEGF expression. In fact, inhibition of VEGF blocks angiogenesis induced by either bFGF[31] or BMP-2[32] and induction of primary osteoblast differentiation by BMP-7 (OP-1)[33] or bone formation by BMP-4[28]. VEGF affects chemotaxis, proliferation, survival and activity of several cell types, including endothelial cells[34], osteoblasts[15,16,19,23,24,35] and osteoclasts[36-38].

Consistent with the fact that endogenous VEGF is important for normal bone repair, exogenous VEGF can promote angiogenesis and bone formation in mouse femur fractures and rabbit radial critical-sized defects[19], and can synergize with BMP-4[28]. VEGF treatment has been shown to increase bone blood flow in radial fractures[27] and during tibial distraction osteogenesis[39]. Finally, VEGF can increase bone formation and decrease bone resorption in intact rabbit femurs[40].

VEGF can stimulate endothelial cells to synthesize osteogenic factors[42,43] and thus indirectly promote bone formation. In addition, osteoblasts make and respond to VEGF[19,24,29,32,33,35,43,46-49]. Many pro-osteogenic factors stimulate VEGF production by osteoblasts[32,33,35,43-47], and VEGF receptor expression is regulated during osteoblast differentiation[35,48,50]. VEGF can induce osteoblast chemotaxis, proliferation, differentiation, and cAMP production[19,24,35,48,49]. VEGF stimulates bone formation in organ cultures and *in vivo* due, at least in part, to direct effects on osteoblasts[24,40]. Inhibition of VEGF impairs *in vitro* osteoblast differentiation[19] and bone growth *ex vivo*[24].

VEGF can also have direct effects on bone-resorbing osteoclasts. In both wild-type and osteopetrotic (op/op) mice, VEGF regulates normal osteoclastic resorption during endochondral ossification[24-26,36,37] by affecting osteoclast recruitment, survival, and activity[36-38,51] and differentiation[36]. While VEGF is likely involved in lamellar bone remodelling in mouse fractures[19], VEGF may have a different role in resorption in intact rabbit femurs[40].

Inhibiting endogenous VEGF or adding exogenous VEGF can also alter cartilage tissue through effects on chondrocyte apoptosis and differentiation and cartilage resorption[24,25-26,28,29,37,52]. These *in vivo* effects of VEGF on cartilage may be indirect through its stimulation of vascularity and cartilage resorbing cells[24,25,28,37]. However, VEGF treatment induces phosphorylation of VEGF receptors in hypertrophic chick chondrocytes, suggesting that chondrocytes may respond directly to VEGF[53].

Summary

Many factors are involved in the communication between osteoblasts and endothelial cells, albeit in slightly different ways. For example, BMP-2 stimulates[54] and VEGF inhibits[23] osteoblast apoptosis, yet both factors stimulate osteoblast differentiation. Simlarly, bone morphogenetic proteins promote commitment of mesenchymal cells to the osteoblast lineage, and many other factors, including VEGF, promote osteoblast differentiation. Those factors which are able to activate both osteoblasts (bone formation) and endothelial cells (angiogenesis) may prove effective as single agents to promote bone repair.

Angiogenesis plays a key role in bone repair. Several key molecules have been discovered and used in animal models to promote bone repair. New drug delivery systems, gene therapy, and small molecules mimetics[56] may allow for lower effective doses[55] and fewer side effects. As biomarkers and pharmacogenomics are applied to the field of orthopaedics, therapies may become better tailored to individual patients, and the incidence of delayed and non- unions may be decreased. The keys to understanding potential bone repair biotherapeutics may be found in the biochemistry of our bones[57].

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